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13. ABSTRACT (Maximum 200 Words)

This is the final addendum report for a 6 month no-cost extension (9/1/02-2/28/03) of the original 3-year grant awarded to investigate the initial development of tissue engineering strategies for breast reconstruction following tumor resection.

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INTRODUCTION

The cure for breast cancer is a long-term clinical realization. In the meantime, patients continue to undergo mastectomies as a preventative measure against breast cancer or as a means to surgically resect an existing breast cancer. Conventional procedures for reconstructing breast, or other soft tissue defects requiring adipose tissue, involve "robbing Peter to pay Paul". That is, tissue from a donor site on the patient is used to reconstruct the breast mound. Ideally, the reconstructive goal would be to completely avoid using functional tissues, such as muscle, for soft tissue reconstruction. Considering the fact that the general cost of reconstruction is high, in both the monetary and the physical sense, a need exists to reduce costs and develop innovative reconstruction methodologies. The multidisciplinary efforts of bioengineering and materials science, cell biology, and surgical science can interact through the field of tissue engineering to help produce viable adipose tissue solutions for presently limited reconstructive applications in soft tissue augmentation and, ultimately, for incorporation into compound flap tissue for clinical use to increase soft tissue bulk and help create or repair appropriate superficial body contour and shape where well-vascularized soft tissue is needed. Figure 1 depicts the overall strategy.

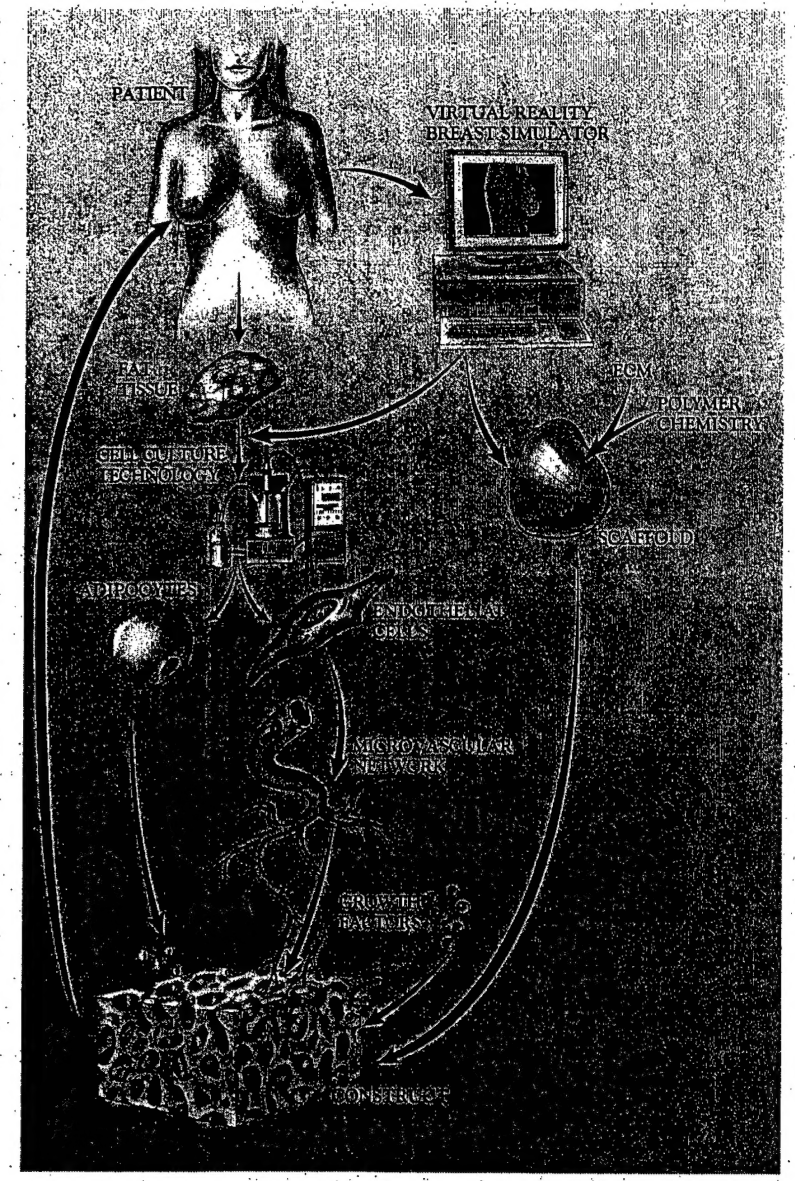


Figure 1. Overall adipose tissue engineering strategy. This grant focuses on the adipose and polymer aspects of the plan. Other grants cover the vascularization issues.

BODY

The specific tasks of the project as originally stated are to:

1. Assess *in vivo* adipose formation in PLGA polymer scaffolds seeded with primary rat preadipocytes and implanted subcutaneously for three and six months. Adipose formation will be assessed histologically using OsO_4 staining.

2. Fabricate breast-shaped PLGA polymer scaffolds using a vacuum-assisted particulate-leaching process and assess polymer architecture. A hemisphere will be used as an initial model breast shape. Pore size distribution, number of pores, and global architecture will be assessed using Hg infusion porosimetry and SEM.

3. Assess feasibility of transferring preadipocyte-seeded, breast-shaped polymer scaffolds as flaps based on an omental vascular pedicle. Conventional tissue transfer and microvascular surgery techniques will be applied to carry out this aim.

This addendum report only covers the 6-month no-cost extension and is intended to be supplementary to the final report submitted and approved last year. The no-cost extension was requested to complete portions of Task 3. Specifically, adipose tissue constructs were just implanted in micropigs at the end of 9/02. The extension was requested to use the final remaining grant balance for costs associated with harvesting, histology, and analysis.

The focus of Task 3 was changed slightly based on the need to develop a large animal model. It was decided that Task 3 could not be conducted appropriately or conclusively in a small animal model. A large animal model does not currently exist for testing clinically translatable adipose tissue engineering strategies for breast reconstruction prior to proceeding to clinical trials in humans. One cannot scale up from small animal models to human studies. Small animal models possess two predominant limitations. One, small animal models cannot be carried out long enough to assess the robustness and persistence of breast restoration strategies. Assessing the maintenance of *de novo* tissue is critical for determining whether tissue is remodeled and resorbed, reaches desired homeostasis, or continues to grow unchecked (i.e., tumor). Second, small animal models do not permit assessment of clinically sized tissue volumes. Tissue regeneration strategies in small animal models are necessarily limited to small volumes that can survive by diffusion and transient neovascularization. This is not the case in humans and large animal models. That is, mass transport issues (i.e., blood supply) are chief design constraints for tissue engineering strategies involving large tissue volumes.

We elected to use Yucatan MicroPigs as a large animal model as they satisfy the criteria for an adipose tissue engineering large animal model listed in Table 1. Approximately 6 months ago, another investigator (W. Morrison) also began to use the pig model for adipose tissue engineering research (personal communication). MicroPigs, specifically, offer the advantages of being a pure breed (less *in vivo* variation), hairless, gentle and tractable, used extensively as a wound healing and cardiovascular models, and have a slow, defined growth rate and desirable size (14-20 kg at sexual maturity/5-6 months and 40-60 kg at adult/12-14 months). Moreover,

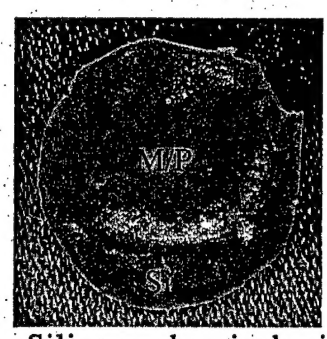
preadipocytes have been successfully cultured from swine in a manner similar to how we have cultured rat preadipocytes.

Table 1: Design criteria for large animal model

Blood vessels must be of sufficient diameter to permit anastamosis using standard microsurgery techniques.
Animal size must be large enough to reflect convection- and diffusion- dependent mass transport.
Animal's tissue structure must permit the harvest, isolation, and culture of preadipocytes.
Animal's anatomy must permit placement of constructs without impairing the animal's mobility or quality of life, and provide protection from mechanical damage due to animal's lifestyle.
Animal's growth rate must permit long-term studies in that the animal must not become too large or reach the end of its natural life expectancy.
Animal's soft tissue and cardiovascular physiology should be similar to human.

In collaboration with a plastic surgeon (E. Beahm), we have conducted preliminary studies of transplanting constructs based on a host blood supply and assessing whether mature constructs can be transferred as a free flap using conventional microsurgical techniques. The epigastric and mammary vessels of male MicroPigs were assessed. The mammary vessels proved to be ideal candidates for construct placement and transfer. Three pigs were utilized in pilot studies. The pigs tolerated the constructs well. Constructs placed in the chest wall were tolerated better and had fewer complications than those placed in the groin region. The groin region (basing constructs on the epigastric vessels) proved to inappropriate because (a) too much mechanical stress on the constructs due to pig wallowing and (b) too much free tissue space in the area, leading to seroma formation. Chest wall placement (based on the mammary vessels) proved ideal and translates directly to the intended strategy for humans.

The pigs were carried out several weeks and the constructs were harvested. As expected, a natural fibrovascular capsule formed around the constructs (as it does with any foreign body). Vessels had remained patent.

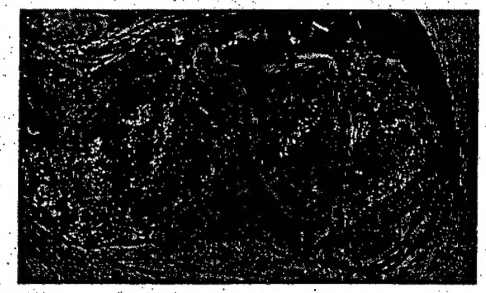


A. Silicone hemispherical constructs were fabricated and filled with a mixture of Matrigel and poly(glycolic acid) polymer non-woven fibers.

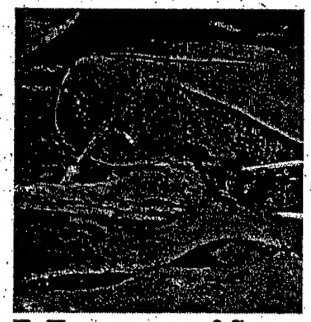
B. Various vessels were isolated using microsurgical technique. Vessel flow was ensured after surgical manipulation via pencil Doppler.

C. Isolated vessel (mammary vessel) was places within the construct.

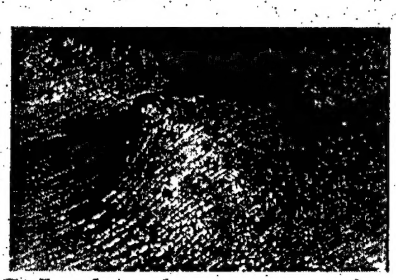
D. A silicone "top" was sewn onto the construct to completely seal the vessel into the Matrigel/polymer interior.



E. Alternative vessel sites were tested (here the epigastric vessels of the groin), but they proved to be too cumbersome and/or they did not provide enough "tissue shielding" from the pigs daily movement (i.e., mechanical stresses due to wallowing).



F. Top aspect of figure shows an open construct. The bottom aspect of the figure shows the construct rotated in proper orientation and placed in the pig's chest. A "breast" mound is formed.



G. Implanted construct at time of harvest.

KEY RESEARCH ACCOMPLISHMENTS

See final report for Year 1-3 accomplishments. Key accomplishments for the 6-month extension include:

- Large animal model development was initiated.
- Pilot studies were successful.

REPORTABLE OUTCOMES

Pilot studies were completed demonstrating the feasibility of using the micropig as an animal model. A competing renewal of this grant was submitted to the DOD such that the large animal model could be further developed, characterized, and employed in adipose tissue engineering studies. The grant, entitled "In vivo porcine test bed for restoring the postmastectomy breast through tissue engineering", was not selected for funding based largely on the fact that the reviewers did not feel that development of a large animal model was within the scope of the Breast Cancer Research program. The large animal model development, deemed by the PI to be

critical for the translation of small animal and bench top research to the human, is currently on hold until additional support can be garnered from extramural or philanthropic sources.

CONCLUSIONS

The results of this grant have been successful in both a young scientific field (tissue engineering) and infant application area (adipose tissue engineering). Feasibility of adipose tissue engineering has been demonstrated, knowledge has been increased, design constraints have been defined, and the next steps required to reach the ultimate goal of a clinically translatable strategy are clear. There are now laboratories in three other countries focused on the area of adipose tissue engineering. Support from the DOD has allowed this laboratory to not only be the first laboratory to publish results but also to remain at the forefront.

REFERENCES:

See final report; no new references based on 6-month extension.